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SNPs affecting serum metabolomic traits may regulate gene transcription and lipid accumulation in the liver

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ARTICLE INFO

Article history:
Received 22 December 2011
Accepted 7 May 2012

Keywords:
Serum metabolites
Hepatic fat
mRNA
Polymorphism

ABSTRACT

Objective. Circulatory metabolites are important biomarkers for many diseases, especially metabolic disorders. The biological mechanism regulating circulatory levels of metabolites remains incompletely understood. Focusing on the liver as the central organ controlling metabolic homeostasis, we investigated the potential function of nine polymorphisms associated with serum metabolomic traits in a recent GWAS.

Materials/Methods. The mRNA levels of the associated genes were measured by real-time PCR and correlated with genotypes in normal liver tissue ($n=42$). Genotype and mRNA data were also correlated with total hepatic lipid content (HLC). Our findings were also compared with the previously published gene expression quantitative traits loci (eQTL) data in the liver.

Results. We found that seven out of nine genes were highly expressed in hepatic tissue, while expression of four genes was significantly or marginally associated with genotypes (*SPTLC3* vs rs168622, $P=.002$; *ACADS* vs rs2014355, $P=.016$; *PLEKHH1* vs rs7156144, $P=.076$; *ACADL* vs rs2286963, $P=.068$). The SNP rs168622 at the *SPTLC3* locus was also significantly correlated with HLC ($P=.02$). HLC was significantly correlated with *FADS1* ($r=-0.45$; $P=.003$) and *ETFDH* ($r=0.33$; $P=.037$) expression. When compared with published eQTL data, SNPs in *SPTLC3*, *ACADS*, *ELOVL2* and *FADS1* were also in strong linkage disequilibrium ($R^2\geq0.41$, $D'\geq0.96$) with eQTLs significantly affecting expression of these genes ($P\leq1.74\times10^{-5}$).

Conclusions. Our study suggests that genetic variants affecting serum metabolites levels may play a functional role in the liver. This may help elucidate the mechanism by which genetic variants are involved in metabolic diseases.

1. Introduction

The dysregulation of the homeostasis of metabolism is involved in the pathogenesis of many diseases. It has long been recognized that the deviation of the circulatory levels of lipids, carbohydrates or amino acids from a normal range is highly correlated with various metabolic perturbations. Understanding the mechanism underlying the quantitative regulation of the circulatory metabolites is of critical importance to delineate the disease pathogenesis.

Abbreviations: SNP, single nucleotide polymorphism; GWAS, genome-wide association studies; HLC, hepatic lipid content; eQTL, expression quantitative trait loci; LD, linkage disequilibrium; FADS1, fatty acid desaturase 1; ELOVL2, ELOVL fatty acid elongase 2; ACADS, acyl-CoA dehydrogenase C-2 to C-3 short chain; ACADM, acyl-CoA dehydrogenase C-4 to C-12 straight chain; ACADL, acyl-CoA dehydrogenase, long chain; SPTLC3, serine palmitoyltransferase, long chain base subunit 3; ETFDH, electron-transferring-flavoprotein dehydrogenase; SLC16A9, solute carrier family 16, member 9; PLEKHH1, pleckstrin homology domain containing, family H; TBP, TATA box binding protein.

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http://dx.doi.org/10.1016/j.metabol.2012.05.004

A recent GWAS was performed for 163 metabolite traits including amino acids, sugars, acylcarnitines and phospholipids in serum measured by ionization tandem mass spectrometry. This study revealed nine single nucleotide polymorphisms (SNPs) located within or around *FADS1*, *ELOVL2*, *ACADS*, *ACADM*, *ACADL*, *SPTLC3*, *ETFDH*, *SLC16A9* and *PLEKHH1* genes which were associated with population variability of serum levels of different metabolites [1]. This integrated metabolomic and genomic approach has significantly furthered our understanding of the key pathways involved in the regulation of human metabolism. However, questions still remain in which organ or tissue these genes and pathways mainly function, and how the function of genes and pathways in one tissue affects the metabolic signatures in the circulatory system. More importantly, whether these GWAS-identified variants and the nearby genes are causative to the alteration of the metabolite traits is largely unknown. Addressing these questions will not only ravel the detailed mechanism involved in the regulation of metabolism in humans, but also help define diagnostic markers and therapeutic targets for various diseases.

Given the central role of the liver in controlling the metabolic homeostasis of carbohydrates, lipids and amino acids in the body, we hypothesize that the regulation of metabolite levels in the blood may be largely attributable to the genes and metabolic pathways in the liver. To test this hypothesis, we investigated the potential role of the aforementioned 9 genetic variants [1] in modulating the gene expression in the liver using tissue samples from healthy donors. Furthermore, since most of these genes are involved in lipid metabolism, and hepatic lipids are one of the major forms of energy storage in the human body, we also tested the relationship between these genes/variants and the lipid accumulation in the tissue samples.

2. Methods

Detailed methods and materials were included as a supplementary appendix.

2.1. Liver tissue samples

Liver tissue samples from 42 healthy Caucasian donors were used in this study. Purdue University and the University of Chicago IRBs have approved their use for the purpose of this study.

2.2. RNA preparation and quantitative real-time PCR

Total RNA was extracted from the liver tissues and real-time PCR was used to quantify the mRNA expression of each gene.

2.3. Genotyping

SNPs were genotyped using 5′ nuclease allelic discrimination TaqMan® SNP genotyping assays (Applied Biosystems, Foster City, CA) (Table S1).

2.4. Hepatic lipid extraction and quantification

Total lipids were extracted from 50 mg of liver tissue using a hexane:isopropanol (3:2) solvent mixture as described in our previous study [2].

2.5. Data analysis and statistics

SNP genotypes were associated with mRNA or hepatic lipid levels using t test. Correlations between mRNA and lipid levels were performed using Pearson correlation.

2.6. Comparison between our findings and previously published eQTL data

A previously published liver eQTL database [3] was searched for the genes investigated. Linkage disequilibrium levels between investigated SNPs and significant *cis*-eQTLs were assessed.

3. Results

3.1. Total lipid extraction and quantification

Total lipid content was quantified in 42 normal livers. The median total lipid content in these livers was 2.9% (range 1.1%–7.9%). Population variability (Coefficient of Variation, CV) in total lipid content was 48.5% (data not shown).

3.2. Association between SNPs and gene expression and hepatic lipid content (HLC)

Seven out of the nine genes investigated were highly expressed in the liver tissue. The mRNA levels of two genes (*ACADM* and *SLC16A9*) were not detectable or quantifiable. We found a significant association between rs168622 and increased *SPTLC3* gene expression (Fig. 1A). The same SNP was also nominally associated with increased total hepatic lipids, but was not significant after Bonferroni correction (Fig. 1G). SNP rs2014355 was also associated with increased *ACADS* mRNA levels (Fig. 1B). Another two variants rs7156144 and rs2286963 were marginally associated ($p<0.1$) with increased mRNA levels of *PLEKHH1* and *ACADL*, respectively (Fig. 1C and D, respectively). No significant correlation was found between rs174547 and rs8396 and either the corresponding mRNA levels or total lipids (data not shown).

3.3. Association between hepatic lipid content and gene expression levels

Hepatic lipid content was significantly correlated with decreased *FADS1* but increased *ETFDH* gene expression (Fig. 1E and F, respectively). The association between *FADS1* gene expression and HLC remained significant after Bonferroni correction.

3.4. Relationship between the SNPs investigated and eQTLs regulating these genes

Statistically significant *cis*-eQTLs were found in *ELOVL2*, *ACADS*, *SPTLC3* and *FADS1* genes. Interestingly, these eQTLs were physically close to and in strong LD with the SNPs investigated in our study (Table 1).